Antagonism Between *Beauveria bassiana* and Imidacloprid When Combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) Control

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ABSTRACT Imidacloprid and the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin are both used to control the whitefly *Bemisia argentifolii* Bellows & Perring. We tested whether the two control strategies acted additively, synergistically, or antagonistically when combined for whitefly control. We found antagonism in that *B. bassiana* inhibited the effectiveness of imidacloprid. When *B. bassiana* was combined with imidacloprid, insect response was either less than or similar to (depending on *B. bassiana* rates) that when imidacloprid was used alone. Adding imidacloprid to *B. bassiana* treatments always increased mortality, but the increase was less than additive. *Beauveria bassiana* spore germination and colony formation were not inhibited by imidacloprid in vitro, and *B. bassiana* did not adsorb or degrade imidacloprid in a tank mix. We hypothesize that *B. bassiana* caused a behavioral response that reduced insect feeding and uptake of imidacloprid.

KEY WORDS *Beauveria bassiana*, *Bemisia argentifolii*, imidacloprid, whiteflies, microbial control, insect pathogens

IMIDACLOPRID, A CHLORONICOTINYL analog of nitromethylene insecticidal compounds, is highly effective against the whitefly *Bemisia argentifolii* Bellows & Perring in many crops (Mullins 1993, Palumbo et al. 1994, Stansly et al. 1998). *Beauveria bassiana* (Balsamo) Vuillemin is an entomopathogenic fungus that can be effective when used as a foliar spray for whiteflies in vegetables (El-Bessomy et al. 1997, Wraight et al. 1998, Zaki 1998, Liu et al. 1999, Wraight et al. 2000). This fungus is usually applied as a spray mixture that contains spores. The spores infect whiteflies through the cuticle, and because nymphs are sessile on the leaf, spores must come into direct contact with the nymph. Thus, obtaining thorough coverage to the underside of leaves, where the insects occur, is imperative to achieving successful control.

Prabhaker et al. (1997) have been able to select for imidacloprid resistance in laboratory populations of *B. argentifolii.* The insect colony originally came from the Imperial Valley, CA, suggesting that at least some field populations of this insect have the genetic reserve to develop resistance if the insecticide is not properly managed. Imidacloprid is often applied as a soil drench at planting but its effectiveness declines during the season, and control is sometimes needed again late in the season. Growers are sometimes tempted to make a second application of imidacloprid at this time, but such practice is not recommended because it may increase selection for resistance. *Beauveria bassiana* could be applied instead, reducing the temptation to make multiple applications of imidacloprid. Alternatively, *B. bassiana* and imidacloprid could be mixed and applied as a foliar spray to control nymphs. The advantage to spray applications is that they can be made after planting, allowing growers to wait and determine whether they need to make an application.

Combining these two pesticides would be most effective if they acted synergistically. Synergistic effects between imidacloprid and *B. bassiana* have previously been reported for termites (Boucias et al. 1996, Ramakrishnan et al. 1999) and the citrus root weevil (Quintela and McCoy 1997, 1998), but not for whiteflies. Using potted cantaloupe, *Cumumis melo* L., plants infested with whiteflies, we tested two application strategies to determine whether imidacloprid and *B. bassiana* acted synergistically, antagonistically, or additively. For the first application method, imidacloprid and *B. bassiana* were mixed in the tank before being sprayed on early third instars. For the second method, imidacloprid was applied as a soil drench to young plants that were later infested with whiteflies, and then subsequently sprayed with *B. bassiana.*

Materials and Methods

For all spray applications, we used a laboratory spray chamber (DeVries MFG, Hollandale, MN) with 1.81 kg/cm^2 of pressure, three hollow cone nozzles (TeeJet TXVS-6, TeeJet Spraying Systems, Wheaton, IL), and a boom speed of 4.8 km/h. This system applied 280 liters/ha. The nozzles were arranged to optimize coverage to the undersides of the plants using a central nozzle with two more nozzles on drops to the

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Table 1. Nine treatment combinations tested in each experiment

Imidacloprid application rate	*B. bassiana* application rate		
	0	0.5× field rate	1× field rate
0	Control	½ rate fungus	Full rate fungus
0.5× field rate	½ rate imidacloprid	½ rate of each product	Full rate fungus + ½ rate imidacloprid
1× field rate	Full rate imidacloprid	½ rate fungus + full rate imidacloprid	Full rate of each product

The design is a 3 × 3 factorial. The field rate for *B. bassiana* was 5×10^{12} conidia/ha. The field rate for imidacloprid was 0.84 ml (AI)/ha when applied as a foliar spray, and 280.8 g (AI)/ha when applied as a soil drench (based on label recommended rates).

sides. The drop nozzles ran near the level of the ground but were directed upward toward the undersides of the leaves.

Comparison of Foliar Combinations. Cantaloupe plants were planted in 10-cm-diameter pots and maintained at ≈25°C in a walk-in growth chamber. All leaves, other than the first three true leaves, were pinched off as they emerged. Plants were infested with whiteflies 21 d after planting by exposing them to adults for 3 d, after which time the adults were removed. Fourteen days later, 40 third instars were marked on each of the two youngest leaves per plant by placing a small black ink spot on the leaf next to each one. Plants were sprayed using the 3 × 3 factorial design described in Table 1. The field application rates were 5×10^{12} conidia/ha for *B. bassiana* (unformulated GHA strain, Mycotech, Butte, MT) using a concentration of 1.75×10^{11} conidia/liter, and 0.84 ml imidacloprid/ha (Provado 1.6 F, Bayer, Kansas City, MO) using 0.003 ml Provado/liter water.

To get fungal conidia into aqueous suspension we used 0.01% Silwet L-77 (Loveland Industries, Greeley, CO) as a surfactant. Silwet L-77 (0.01%) was also added to the controls and the imidacloprid-only treatments. The density of conidia on the leaves was measured by pinning plastic microscope coverslips onto the top and bottom surface of the oldest leaf of each plant before spraying. A straight pin was stuck directly through the middle of the cover slip and the leaf, and into a small piece of foam rubber on the other side. After the plants were sprayed, each coverslip was examined under a microscope (400× magnification) to determine the number of spores per square millimeter as described by Wraight et al. (2000).

The plants were then returned to a walk-in growth chamber maintained at between 21 and 28°C. A humidifier was continuously run in the chamber after spray applications. Humidity levels reached saturation for ≈8 h per night. Low humidity levels during the day ranged from 50 to 60% RH. Survivorship of the marked nymphs was determined 7 d after treatment. Nymphs that were flattened and shriveled were considered dead. *Beauveria bassiana* frequently turns whitefly nymphs a deep red-brown color; therefore, any nymphs so colored were also counted as dead.

The entire experiment was repeated on three different dates. For each experimental run, we used six plants per treatment combination and 80 insects per plant. Viability of *B. bassiana* conidia was determined on each application date by plating the spores on Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY). After 18–20 h, the spores were observed under 400× magnification. Five hundred spores were observed and the proportion that had germinated was determined.

Combining Soil Applied Imidacloprid with Foliar *B. bassiana.* Spray applications of imidacloprid can control whitefly nymphs, but systemic applications are more commonly used for vegetables and are effective against both adults and nymphs. Thus, we tested imidacloprid as a soil drench combined with a later spray application of *B. bassiana.* Again, the experiment was as described in Table 1. We used six plants per treatment in each experimental run, and the entire experiment was repeated twice.

Standard cantaloupe planting density is one plant every 0.3 m using beds that are 2 m wide (16,151 plants/ha), and the label recommendation for soil applications of imidacloprid is 280.8 g (AI)/ha^{-1}, or 0.017 ml (AI) per plant. Using this rate, we treated plants 21 d after planting. For the full field rate we watered each plant with 0.09 ml Provado in 30 ml of water 4 d before the plants were infested with whiteflies. Adult whiteflies were maintained on these plants throughout the rest of the experiment by adding new adults every 3–7 d as needed. Plants were sprayed with *B. bassiana* (as described above) 16 d after the first whitefly infestation (20 d after imidacloprid treatments). The plants were maintained using the same temperature and humidity regimes described for the previous experiment. Seven days after the *B. bassiana* treatment we determined the number of live, immature whiteflies (by stage) on the two youngest leaves of each plant. Leaf size was then determined using a Li-300 area meter (Li Cor, Lincoln, NE) and the density of immatures (number/cm^2) was calculated.

Statistical Analyses of Experiments. Both experiments described above were two-way analyses of variance (ANOVA) using imidacloprid and *B. bassiana* applications as the main effects. Each plant was considered a replicate (18 replicates for the first experiment and 12 for the second). We also included the date of each experimental run in the ANOVA table as a main effect so that we could account for variation between runs. In addition to testing for main effects (imidacloprid and *B. bassiana* effects), we tested whether the interaction between imidacloprid and *B. bassiana* was significant. For the spray tank mix experiment, the dependent variable was mortality of whitefly nymphs. For the second experiment, we tested whether systemic imidacloprid and *B. bassiana* sprays affected egg and nymphal density. Although

egg and nymphal densities do not allow us to differentiate between effects on adults and immatures, it does give us a measure that is relevant to field control in areas where large numbers of whiteflies are immigrating.

Testing for a Direct Effect of Imidacloprid on *B. bassiana*, and Vice Versa. During one experimental run of the tank mix bioassay, we tested whether imidacloprid might affect germination of *B. bassiana* conidia. We sampled spores from the tank mix after all the spray applications had been completed (≈1 h) and plated 0.1 ml onto SDAY. We tested the three treatments that had *B. bassiana* at 0.5 times the field rate and imidacloprid at 0, 0.5, and 1 times the field rate. The spores were incubated for 20 h at 25°C and then observed for germination at 400×. Three plates were used for each treatment, and the number of spores that had germinated in the first 100 observed was recorded for each plate.

The effect of imidacloprid on spore germination and colony formation was also tested by soaking the spores for 1 h in imidacloprid at the same concentrations used in the tank mixes. Spores were soaked in water as a control. Both spore suspensions contained 0.01% Silwet L-77. A series of 10-fold dilutions of each spore mixture were then made, and the dilutions were plated on SDAY and the number of colony forming units (cfu) was determined after incubating the plates for 3 d at 25°C. This assay was replicated six times. We compared the number of colony forming units in the original control mixture with that for spores soaked in imidacloprid.

Chemical analysis was done to determine if *B. bassiana* adsorbs or degrades imidacloprid when the two materials are mixed (e.g., as would occur in a tank mix). A primary standard solution (100 mg/liter) was prepared by dissolving technical grade imidacloprid in 50 ml of ethanol. This stock standard solution was diluted (1:10) with methanol to obtain a working standard solution (10 mg/liter). Analytical standards (0.1–10 mg/liter) for high-performance liquid chromatography (HPLC) calibration were prepared by dilution of the stock standard with methanol. For analysis of tank mixtures, *B. bassiana* was added to formulated imidacloprid (0.003 ml Provodo/liter water) at a rate of 1.75×10^{11} conidia/liter, yielding the same concentrations used in the tank-mix bioassay. Imidacloprid was quantified before and after the addition of *B. bassiana.* After *B. bassiana* was added, the mixture was centrifuged at 4,000 × *g* for 15 min to remove *B. bassiana* spores from the suspension. An aliquot was obtained from the supernatant and subjected to HPLC analysis. If *B. bassiana* does not adsorb imidacloprid, we would expect the concentration to be the same in the supernatant as it was in the solution before the fungus was added. To remove any imidacloprid that might remain in the pellet, but that was not adsorbed, the spores were washed three times by removing the supernatant, mixing the spores back into suspension on an electric vortex mixer, and centrifuging again. The final pellet was brought back to the initial volume by adding sterile, deionized water and resuspending with a vortex mixer. Then an aliquot was subjected to HPLC analysis.

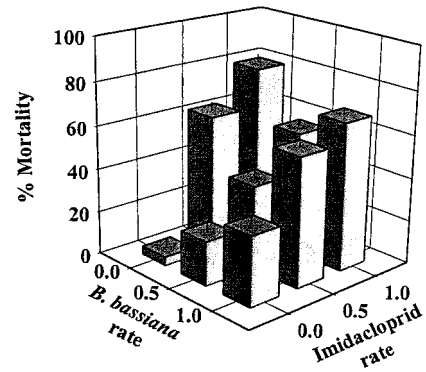

Fig. 1. Whitefly mortality when imidacloprid and *Beauveria bassiana* were mixed in the spray tank and applied foliarly. Rates were 1 and 0. 5 times the recommended field rates of 5×10^{12} conidia/ha for *B. bassiana,* and 0. 84 ml imidacloprid/ha.

All solvents used were HPLC grade from Mallinckrodt (Paris, KY). Water was glass-distilled and further purified through a Millipore Mili-Q water purifier. Technical-grade imidacloprid was obtained from Chem Service, West Chester, PA. The HPLC analyses used were a modification of the methods of Baskaran et al. (1997), as follows. All standard samples, imidacloprid solutions, and *B. bassiana* suspensions were analyzed on a Hewlett–Packard 1090 Series II HPLC equipped with a diode array detector, programmable variable-wavelength UV detector, an autoinjector, and a Vectra Chemstation (Agilent Technologies, Austin, TX). All analyses were performed on a Waters reversed phase, C18, dimethyloctadecylsilyl bonded amorphous silica-methyl alcohol, Bondapak column (300 by 3.9 mm i.d., 10 μm particle size) (Millipore, Milford, MA), using a mobile phase of acetonitrile-water (20:80, vol:vol) at a flow-rate of 1.5 ml/min. The detection was performed at 270 nm and 0.02 aufs. Sample injection volume was 10 μl.

Results

Comparison of Foliar Combinations. The main effects of *B. bassiana* and imidacloprid were both highly significant (*B. bassiana* $F = 9.67$; df = 2, 148; $P < 0.0001$; imidacloprid $F = 108$; df = 2, 148; $P < 0.0001$). When *B. bassiana* and imidacloprid were sprayed in combination, the effect was equal to or less than that of imidacloprid used alone (Fig. 1). The interactive effect was significant ($F = 9.47$; df = 2, 148; $P < 0.0001$), i.e., the effectiveness of imidacloprid depended, in part, on whether *B. bassiana* was present. Spraying *B. bassiana* and imidacloprid together decreased the effectiveness of imidacloprid (Fig. 1). When combined with imidacloprid, the full field rate of *B. bassiana* resulted in higher mortality than the half-rate, but the amount of control achieved was still only equal to or less than that achieved by imidacloprid alone.

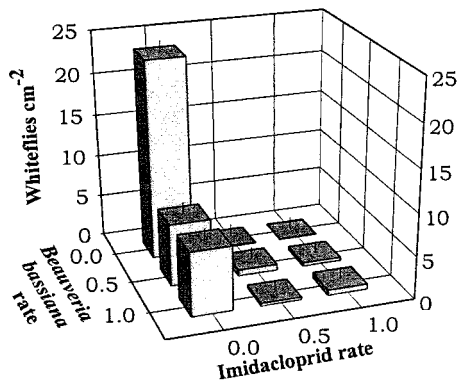

Fig. 2. Density of whitefly immatures (eggs and nymphs) when imidacloprid was applied as a soil drench and *Beauveria bassiana* was applied as a foliar spray. Rates were 1 and 0. 5 times the recommended field rates of 5×10^{12} conidia/ha for *B. bassiana*, and 280. 8 g imidacloprid/ha.

Combining Soil Applied Imidacloprid with Foliar *B. bassiana*. Systemic imidacloprid and foliar applications of *B. bassiana* had significant effects on whitefly density (*B. bassiana* $F = 11.76$; df = 2, 93; $P < 0.0001$; imidacloprid $F = 70.19$; df = 2, 93; $P < 0.0001$). Although the interactive term in the ANOVA was significant ($F = 13.42$; df = 2, 93; $P < 0.0001$), imidacloprid in this experiment was so effective alone that adding *B. bassiana* was of little consequence. We did observe a slight increase in density of immatures when *B. bassiana* was added. We tried using imidacloprid drenches at rates as low as 1/32 the field rate, but still obtained almost complete control of whitefly immatures (R.R.J., unpublished data).

Tests for Direct Effects of Imidacloprid on *B. bassiana*, and Vice Versa. Imidacloprid was found to have no direct effect on *B. bassiana* viability. Conidia soaked in formulated imidacloprid had a mean viability of 97.3 ± 1.4% (mean ± SE) at the high field rate, 98.0 ± 0.7, SE% at the low field rate, and 97.0 ± 1.4, SE% in the absence of imidacloprid. Colony formation also was not affected by imidacloprid: cfu's were 0.90 ± 0.21 times that of the control (no imidacloprid) for the high field rate of Provado, and 1.37 ± 0.14 for the low field rate.

High-performance liquid chromatography showed no significant quantitative changes in imidacloprid concentration after *B. bassiana* had been added. There was no significant difference in the quantity of imidacloprid in the supernatant of the mixture compared with imidacloprid alone or with the imidacloprid + *B. bassiana* mixture, and no imidacloprid was detected in the resuspended pellet. Thus, it appears that there was no binding, adsorption, or chemical degradation of imidacloprid by *B. bassiana* spores.

Discussion

We conclude that although imidacloprid can increase the effectiveness of *B. bassiana*, the levels of control achieved are not any greater than those achieved using imidacloprid alone, and in fact are sometimes lower than when imidacloprid was used alone. The mechanism by which a fungal pathogen could inhibit a chloronicotinylate analog is uncertain. We tested to see if imidacloprid inhibited spore germination and colony formation in the fungus, but no effects were seen. Gardner and Kinard (1998) also found no response of either conidial germination or mycelia growth to imidacloprid at 0.001–100 ppm. Imidacloprid was at 3 ppm in our tank mix.

We also tested to see if imidacloprid might be adsorbed by *B. bassiana* conidia. Such an interaction might reduce the effective concentration of imidacloprid. However, no such adsorption occurred. Others have tested combinations of entomopathogenic fungi and imidacloprid against a variety of insects. Steinkraus (1996) and Brown et al. (1997) found that the combination of imidacloprid and *B. bassiana* yielded greater control of adult tarnished plant bugs in cotton over the use of either treatment alone. The effects they saw were no more than additive, but synergistic effects have been reported for other insects (Boucias et al. 1996, Quintela and McCoy 1997, Ramakrishnan et al. 1999). The synergy found in these studies could all be attributed to behavioral changes in the insects. For example, imidacloprid reduces movement of the citrus root weevil, and this lack of movement in the soil may prevent the insects from dislodging fungal spores, as might otherwise occur (Quintela and McCoy 1998). And termites groom each other, removing fungal spores and preventing infection, but imidacloprid retards this behavior (Boucias et al. 1996).

Thus, it seems likely that a behavioral response occurred in this case as well. Whitefly nymphs have rather limited behavior, but it is possible that *B. bassiana* reduced the feeding rate of nymphs, and because imidacloprid is taken up systemically through the plant, reduced feeding would reduce the exposure rate. Nauen et al. (1998) report that adult whiteflies can survive 2 d of starvation, but we do not know how starvation or reduced feeding rates directly impact the nymphs. Such a behavioral response might also explain why a greater antagonistic response was seen in the tank mix trials than when imidacloprid was used in a soil drench followed by later spray applications of *B. bassiana.* In the latter case, the insects were exposed to imidacloprid for several days before being exposed to *B. bassiana.* With this application method, the full effect of imidacloprid might have already occurred, or the insects might already have achieved a full effective dose before *B. bassiana* was applied. The lack of an antagonistic response here could also be explained by the fact that imidacloprid was much more effective against whiteflies in the soil applied treatments.

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